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# ALIMENTARY TRACT

# Natural Gastric Infection With *Helicobacter pylori* in Monkeys: A Model for Spiral Bacteria Infection in Humans

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**Background/Aims: There is no generally accepted model for *Helicobacter pylori* infection in humans. The aim of this study was to examine the natural history and effect of treatment in rhesus monkeys and sequentially define the immune response to *H. pylori* in relation to treatment. Methods: Infection and gastritis were graded blindly by histological analysis and culture of biopsy specimens harvested during gastroduodenoscopies in 26 anesthetized colony-bred monkeys. Plasma *H. pylori*–specific immunoglobulin (Ig) G levels were determined by enzyme-linked immunosorbent assay. Results: *H. pylori* and *Gastrospirilum hominis*–like organisms were present in 13 and 9 monkeys, respectively; 3 animals harbored both organisms, whereas 4 monkeys were not infected. Gastritis score was ≤1.5 in animals uninfected or infected only with *G. hominis*–like organisms and ≥2.0 in all *H. pylori*–infected animals. IgG ratios were ≥0.5 in 12 of 13 *H. pylori*–infected animals and in 2 of 13 *H. pylori*–negative animals ($P < 0.001$). One monkey became infected with *H. pylori* during the observation period, with concurrent increase of gastritis and plasma IgG levels. In untreated animals, infection, gastritis, and plasma IgG levels remained unchanged over 7–15 months. Triple therapy eradicated *H. pylori* at 6 months in 4 of 6 animals while suppressing gastritis and plasma IgG levels. Conclusions: Rhesus monkeys harboring *H. pylori* are persistently infected and have gastritis and elevated specific IgG levels, all of which may respond to appropriate therapy, whereas *G. hominis* infection is associated with little inflammation.**

Since the first report of its isolation in 1983,[1] *Helicobacter pylori*, previously named *Campylobacter pylori*, has been implicated in the pathogenesis of gastritis[2] and duodenal ulcer disease[3] and as a risk factor for adenocarcinoma and lymphoma of the stomach.[4–6] The diagnosis of this infection has been primarily based on identification of the organisms in gastric mucosal biopsy specimens, although noninvasive methods such as the urea breath test[7] and detection of specific serum antibodies[8] have facilitated larger epidemiological studies. Another type of spiral bacterium has also been described in the stomach of patients with gastric cancer[9] and in patients with upper gastrointestinal complaints.[10,11] The provisional name of this bacterium is *Gastrospirillum hominis*, and it has been suggested that it also could be a pathogen.[12,13] More recently, polymerase chain reaction (PCR) amplification of 16S ribosomal RNAs has indicated that this organism belongs in the *Helicobacter* genus, and it appears to be closer to *Helicobacter felis* than to *H. pylori*.[14] Although the name *Helicobacter heilmannii* was initially proposed by these investigators, their examination of different clones led them to conclude that there are probably many species of these bacteria and that it is premature to propose an official name.[14]

Despite the number of observations in humans, we still lack direct experimental evidence for a causal relation between these bacterial infections and subjective symptoms as well as pathological findings. This is caused, in part, by ethical considerations in humans and by the absence of an accepted animal model.

The *Helicobacter* genus presently comprises nine species that have been isolated from different animal species,

*Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; FET, Fisher's Exact Test; GHLO, *Gastrospirillum hominis*–like organisms; PCR, polymerase chain reaction.



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including primates.[15] The *Helicobacter* species that have been isolated from naturally infected ferrets, cats, and cheetahs share many properties with *H. pylori* observed in humans,[16–18] although important differences have also been noted. In nonhuman primates, *H. pylori*–like organisms isolated from baboons, pigtailed macaques, and rhesus monkeys have been found to be morphologically and biochemically similar to *H. pylori* isolated from humans.[19–21] However, the DNA homology of the organisms isolated from pigtailed macaques with other members of the *Helicobacter* genus was later found to be <10%.[22] In contrast, *H. pylori*–like organisms isolated from rhesus monkeys have been found to be very similar to human *H. pylori* by all the phenotypic tests that have been applied to date, i.e., total protein profile, antiurease monoclonal antibody, and hyperimmune rabbit antiserum using a 10%–25% linear gradient sodium dodecyl sulfate–polyacrylamide gel electrophoresis system.[21] Furthermore, PCR amplification and partial 16S ribosomal RNA gene sequence analysis has indicated that the rhesus monkey and human isolates are highly homologous.[23a] Taken together, these data strongly suggest that the rhesus monkey isolates that we have grown in vitro are *H. pylori*. In addition, baboons and rhesus monkeys are frequently infected with another gram-negative, urease-positive, tightly coiled spirilla that is identical in morphology to human *G. hominis* by light and electron microscopy.[19,21] Neither human *G. hominis* nor the *G. hominis*–like organisms (GHLO) observed in baboons or rhesus monkeys have been grown in vitro.[24]

Based on these observations, both baboons and rhesus monkeys appear to represent potential models to evaluate the role played by gastric mucosal infection with either of the spiral bacteria found in humans in the production of gastritis and of the associated immune response; these models also permit the evaluation of antimicrobial therapies. However, the genome of *H. pylori*–like organisms isolated from baboons has not yet been characterized, and these animals are bigger and more difficult to handle than rhesus monkeys.

Therefore, the goals of the present study were to determine the natural history of *H. pylori* and GHLO infections in rhesus monkeys; to evaluate the effect of treatment in these animals; and to sequentially define the immune response of infected rhesus monkeys to *H. pylori* in the presence or absence of treatment.

## Materials and Methods

### Animals

Twenty-six domestic, colony-reared, male rhesus monkeys, *Macaca mulatta* (age, 2–5 years; weight, 3–5 kg), were first quarantined for 90 days in individual stainless steel cages in conventional holding rooms of an animal facility approved by the American Association for Accreditation of Laboratory Animal Care and were subsequently kept in similar individual housing. They had not been used in any other research protocol before being included in the present studies. Animals were provided with tap water ad libitum, commercial primate chow, and fruit. After three negative intradermal tuberculin test results, with tests performed at 2-week intervals, animals were released from quarantine. All subsequent studies were performed between 8 AM and noon after an overnight fast.

### Endoscopic Procedures and Biopsies

Each rhesus monkey underwent gastroduodenal endoscopic examination under general anesthesia (atropine sulfate, 0.02 mg/kg intramuscularly followed by ketamine hydrochloride, 10 mg/kg intramuscularly) using a videogastroscope with an outer diameter of 9.8 mm (model 81200; Welch–Allyn, Skaneateles Falls, NY). Between each endoscopy, care was taken to first rinse the endoscopic equipment with water and then disinfect it sequentially with solutions of 2% glutaraldehyde and 95% ethanol. The macroscopic appearance of corpus and antral mucosae was assessed qualitatively but, as previously reported,[21] was not significantly related to any of the other features of infection. In each animal, two pinch biopsy specimens of the gastric mucosa were obtained each from the corpus and the antrum. One of the specimens from each region of the stomach and a specimen obtained from the duodenum were fixed in neutral 10% buffered formalin and routinely processed for light microscopy. Five-micrometer paraffin-embedded sections were processed for H&E and Gram staining and viewed under ×100–×1000 magnification. Initially, Warthin–Starry staining was also performed for identification of spiral bacteria; however, we found that similar accuracy was obtained with H&E and Gram staining and subsequently performed only these preparations. Two additional biopsy specimens were taken each from the corpus and antrum of three *H. pylori*–infected and three GHLO-infected rhesus monkeys. These specimens were fixed in Karnovsky's solution and processed routinely for transmission electron microscopy.[25] Coded ultrathin sections were evaluated to determine the presence and characteristics of *H. pylori* and GHLO using a Philips 400 electron microscope (Philips, Mahwah, NJ) at 80 kV.

### Rating of Infection

Based on the appearance of *H. pylori* and GHLO on histological examination,[21] coded H&E- and Gram-stained sections were scored for intensity of infection at ×1000 using a scale of 0–3 as follows: 0, no bacteria; 1, colonies seen in 1–2 of 10 fields of view; 2, colonies seen in 3–8 of 10 fields; and 3, colonies seen in 9–10 of 10 fields. One biopsy specimen from the corpus and one from the antrum were immediately placed in sterile 0.9% NaCl, kept on ice, coded, and then prepared for culture, smears, and urease assay by homogenization with a sterile ground-glass cone-shaped pestle fitting a tapered 1.5-mL Eppendorf tube. Sterile 1–2-μL loops were filled and streaked on agar plates prepared with Mueller–Hinton media supplemented with 5% sheep blood and incubated at 37°C in sealed chambers with an atmosphere of 90% $N_2$, 5% $O_2$, and 5% $CO_2$. Ringed microscope slides were used for smears and Gram stains of 1-μL aliquots of the homogenate and of colonies (dispersed in sterile saline) that grew within 7 days. *H. pylori* identification was based on (1) pinhead-sized "water-spray" colonies positive for urease[26] and oxidase (Becton–Dickinson, Cockeysville, MD) and catalase (formation of bubbles in 3% $H_2O_2$) activities; (2) presence, in these colonies, of gram-negative curved or "gull-wing" rods; and (3) a kinetic assay showing high urease specific activity (>1 $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$) plus high-affinity substrate binding (Michaelis constant [$K_m$] < 1 mmol/L),[27] in at least one culture from each rhesus monkey.

### Rating of Gastritis

The presence and extent of gastritis was rated independently from the scoring for infection on coded H&E-stained slides using a scale of 0–3, modified from Marshall and Warren,[28] as follows: 0, intact mucosal lining and essentially no infiltration of the lamina propria with lymphocytes and plasma cells; 1, mild increase of mononuclear infiltration localized in upper half of the mucosa; 2, marked mononuclear infiltration extending from the surface to the muscularis mucosae; and 3, presence of polymorphonuclear leukocytes in glands, which was always associated with marked mononuclear infiltration and surface erosions. Duodenal biopsy specimens were evaluated for the presence of gastric metaplasia. Preobservation or pretreatment infection and gastritis scores of 4 of the 26 rhesus monkeys (1 uninfected, 1 *H. pylori* infected, and 2 GHLO infected) have been included in results previously published.[21]

### Measurement of *H. pylori*–Specific Plasma Immunoglobulin G Levels

At each endoscopy, 5 mL of ethylenediaminetetraacetic acid–treated blood was obtained and the plasma was frozen at −70°C. Plasma immunoglobulin G (IgG) levels were determined blindly using a previously described enzyme-linked immunosorbent assay (ELISA) with >95% sensitivity and specificity for human infection.[29,30] In addition, all samples were run a second time using anti-monkey antibody conjugates. In brief, the *H. pylori* antigen used in the ELISA was prepared from bacterial suspensions from five *H. pylori* strains representing a range of antigens. The sonicates from each strain were pooled and diluted in 0.05 mol/L carbonate buffer (pH 9.6) to yield the optimal protein concentration of 10 μg/mL. A 0.1-mL aliquot of this solution was added to each well of a flat-bottomed Immulon 2 plate (Dynatech Laboratories, Alexandria, VA). The screening serum dilutions were 1:800, whereas perioxidase conjugates of goat anti-human (Tago Inc., Burlingame, CA) and anti-monkey (Nordic, Capo Beach, CA) IgG were diluted 1:2000. Results were corrected for day-by-day variation of the ELISA and expressed as optical density ratios. In humans, an IgG ratio > 1.0 has been considered indicative of the presence of anti–*H. pylori* antibodies. All assays were performed at least in duplicate. Tests for possible cross-reactivity of *H. pylori* antibodies had been performed by absorbing serum from *H. pylori*–infected persons who had high values in the IgG ELISA with cells of other enteropathogens.[29] For studies of the time course of infection or of the effect of treatment, all samples collected in a specific animal were run on the same day and were included in the same plate.

### Follow-up Examinations and Treatments

Fifteen of the rhesus monkeys (4 uninfected, 6 *H. pylori*–infected, and 5 GHLO-infected as assessed by histological examination and/or culture) were re-evaluated 7–15 months later by endoscopic biopsies and plasma IgG determinations. In addition, two therapeutic trials were performed in 12 of the infected rhesus monkeys. First, 6 rhesus monkeys (2 *H. pylori*–infected and 4 GHLO-infected) were treated with oral amoxicillin plus metronidazole plus bismuth subsalicylate (7, 7, and 10 mg/kg, respectively, three times daily) diluted in Tang (flavored powder that, when reconstituted with water, produces a fruity drink that rhesus monkeys consume readily; General Foods Corp., White Plains, NY) for 4 weeks; endoscopies and plasma IgG determinations were repeated 1 and 3 months later. Second, 6 other rhesus monkeys infected with *H. pylori* were treated with amoxicillin plus metronidazole plus bismuth subsalicylate (7, 7, and 10 mg/kg, respectively) diluted in 5 mL of sterile water and administered intragastrically twice daily for 10 days; endoscopies and plasma IgG determinations were repeated 1 week and 1, 2, 3, 5, and 6 months after the end of treatment.

### Statistical Analysis

Results are expressed as means ± SEM. A two-way analysis of variance with repeated measures[31] was used to determine the effects caused by type of infection, by time or treatment, or by an interaction among these two factors. This statistical method takes into account that measurements were repeated sequentially in the same animals by establishing a distinction between a factor that classifies the subjects into groups (grouping factor) and a factor for which each subject is measured at all times (within-subject factor). Computer implementation of this statistical method was performed using locally developed programs. Linear correlation coefficients were calculated using SlideWritePlus software (Advanced Graphic Software, Carlsbad, CA). Fisher's Exact Test (FET) and Mantel–Haenszel corrected $\chi^2$ test were performed when appropriate.

## Results

### Prevalence of Gastric Infection as Assessed by Light Microscopy and Culture

In 4 of 26 naive (untreated) colony-reared rhesus monkeys, no spiral bacteria were observed by histological analysis of the biopsy specimens harvested from the corpus or antral mucosae; cultures of other gastric biopsy specimens obtained in the same animals were negative. In 13 other rhesus monkeys, *H. pylori* was observed by

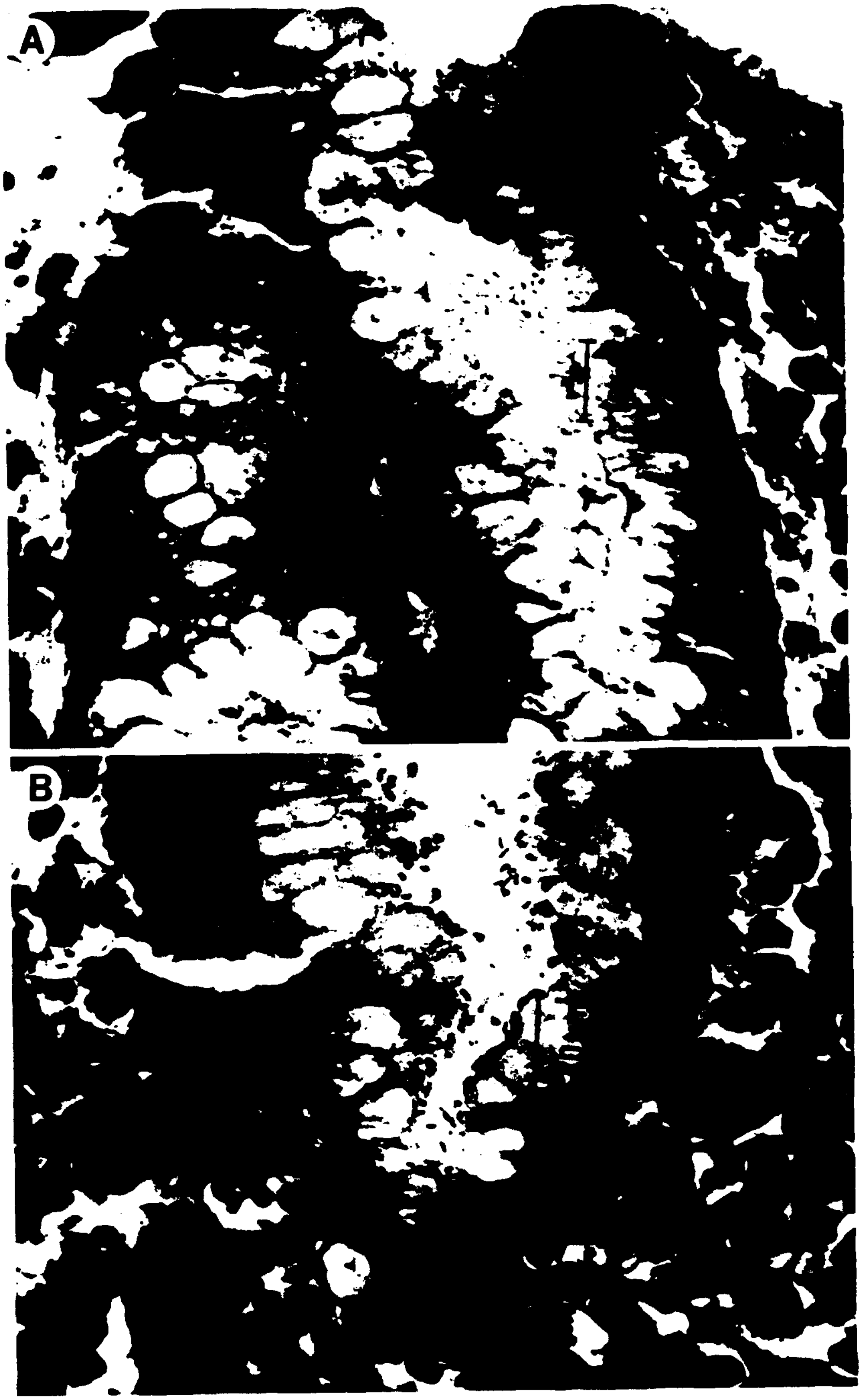


Figure 1. Composite photomicrograph of the (*A*) gastric pit and (*B*) crypt of an *H. pylori*–infected rhesus monkey (H&E; original magnification ×1000). Note the mucous depletion of superficial epithelial cells and the intense mononuclear and neutrophil infiltration.

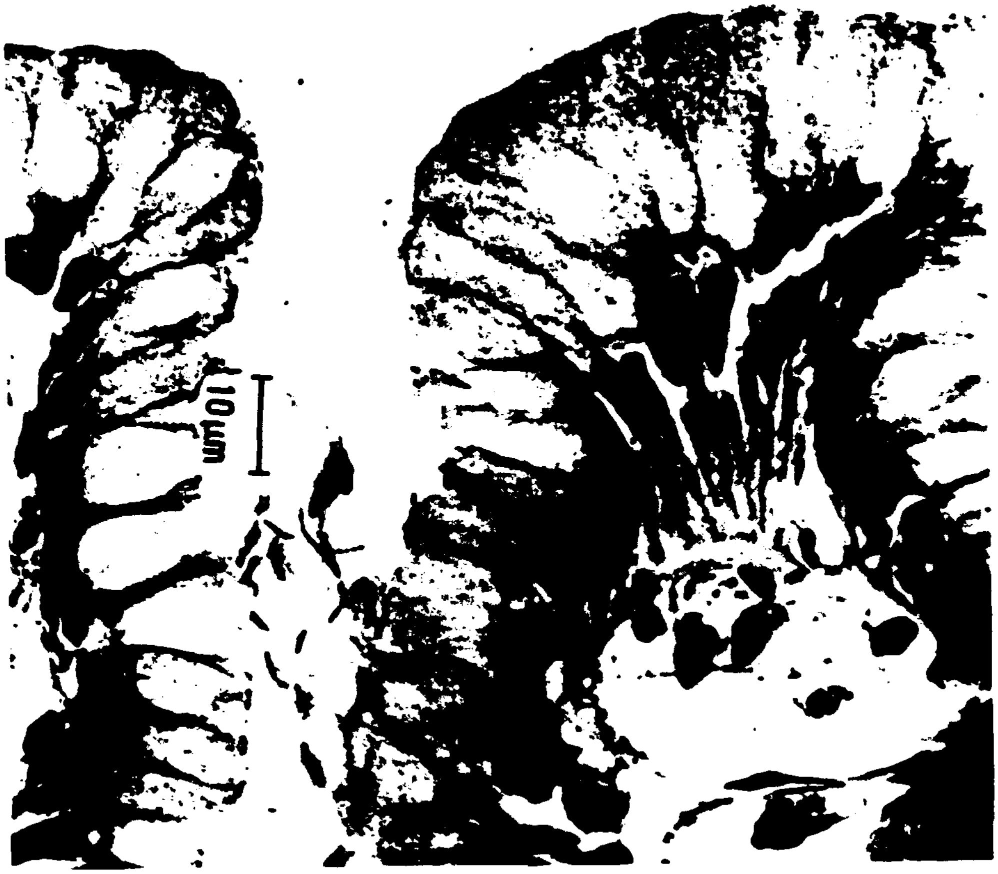


**Figure 2.** Gastric pit of a GHLO-infected rhesus monkey (H&E; original magnification ×1000). Note the absence of neutrophil infiltration and the discrete mononuclear infiltration.

histological analysis in both the corpus and the antrum (Figure 1) (infection score, 1.38 ± 0.30 and 1.85 ± 0.31, respectively), and the characteristic bacterial growth with high-activity production of a urease with tight substrate binding[32] was found in at least one of the specimens in each of these animals. A small number of GHLO also were observed in the corpus of 3 of these rhesus monkeys. In subsequent analyses, we defined as *H. pylori*–infected those animals that had at least one specimen containing *H. pylori* as evidenced by either light microscopy or culture. In the 9 remaining rhesus monkeys, GHLO alone were observed by histological analysis of the specimens obtained in the corpus and, in addition, in the antrum of 6 of these animals (infection score, 2.67 ± 0.16 and 1.89 ± 0.46, respectively) (Figure 2). As has been reported in human studies,[24] no bacterial growth was observed in the many cultures prepared from these specimens, despite the repeated microscopic observation of the clearly visible, well-stained, long (4–7 μm), corkscrew-like, gram-negative organisms in smears of the same specimens. In contrast, *H. pylori* organisms could not be visualized in the smears of similar specimens harvested from *H. pylori*–infected rhesus monkeys, because they are indistinguishable from the ubiquitous tissue fibrils that also are stained by safranin. All biopsy specimens containing GHLO had urease activity, often manyfold higher than the specimens containing *H. pylori*; in two cases, there was enough material to directly evaluate urea binding affinity by spectrophotometric assay. In both cases, the $K_m$ was <1 mmol/L, as was true for the specimens obtained in *H. pylori*–infected animals; accordingly, no differentiation was possible by this criterion. Indeed, the characteristic urease supports the recent argument based on molecular genetics that GHLO belong within the genus *Helicobacter*.[14] Thus, natural infection with *H. pylori* and/or GHLO were common in this population of rhesus monkeys.

### Appearance of Organisms by Transmission Electron Microscopy

*H. pylori* were observed in close proximity to the surface of epithelial cells. In case of heavy infection, very few microvilli were visible and the bacteria appeared to be attached to pedestals similar to those described in

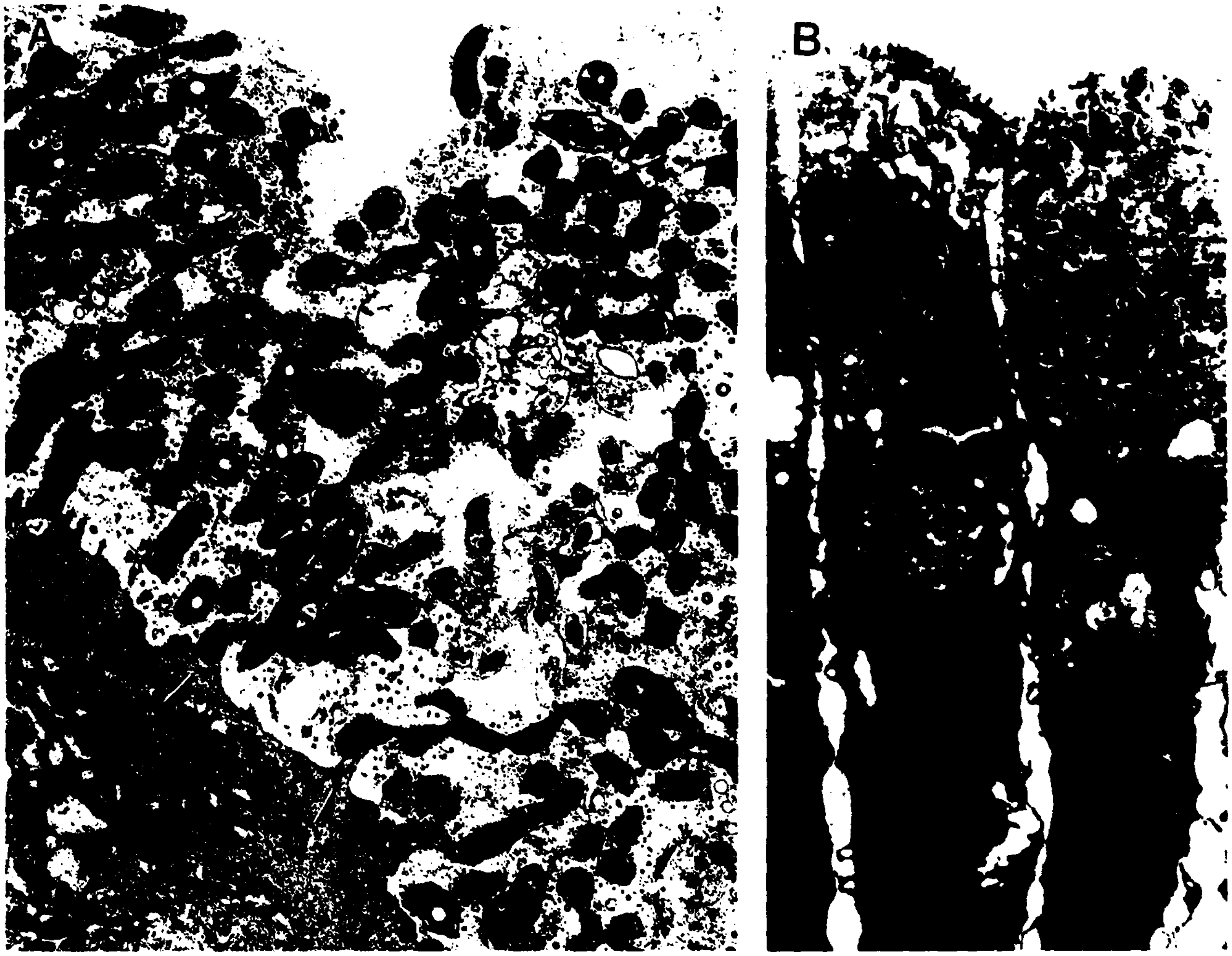


**Figure 3.** Transmission electron micrographs of *H. pylori*. (*A*) Note the virtual disappearance of microvilli, the formation of pedestals (*arrowheads*), and the close association between *H. pylori* and these pedestals (original magnification ×12,000). (*B*) Intact microvilli and intracellular organelles on two superficial epithelial cells, one of which has two *H. pylori* within its cytoplasm (original magnification ×10,000).

humans[33] (Figure 3*A*, arrows). In contrast, microvilli and intracellular organelles were well preserved in areas of minimal infections, and a few *H. pylori* were occasionally present in the cytoplasm of superficial epithelial cells well below the level of the tight junction (Figure 3*B*). GHLO were never observed in close proximity with, or within the cytoplasms of, surface epithelial cells, and the microvilli were always intact (Figure 4). As previously reported,[21] GHLO, but not *H. pylori*, were often seen within the cytoplasm of parietal cells.

## Relationship Between Gastric Inflammation and Infection With Gastric Organisms

For the entire group of 26 animals, inflammation scores of the corpus and antrum were significantly correlated ($r = 0.69$; $P < 0.01$). Therefore, the average of the two scores was subsequently used as an index of the amount of inflammation present in the stomach. All 13 rhesus monkeys infected with *H. pylori*, including 3 animals also infected with GHLO, had mean scores ≥ 2.0 (Figure 5), whereas 3 of 4 apparently uninfected rhesus monkeys had scores ≤ 0.5 ($P = 0.005$; FET, two-tailed). Among the 9 rhesus monkeys infected with GHLO alone, 8 animals had scores ≤ 1.0 (Figure 5); 5 of them had a score of 0 (Figure 5), whereas all 11 rhesus monkeys infected with *H. pylori* alone had scores ≥ 2.0 ($P < 0.001$; FET, two-tailed). Analyzing the data in another way, all 13 rhesus monkeys with mean gastritis scores ≥ 2.0 had detectable *H. pylori* infection compared with 0 of 13 with lower scores ($P < 0.001$; FET, two-tailed). Thus, a mean gastritis score ≥ 2.0 was 100% sensitive and 100% specific for *H. pylori* infection. Finally, gastritis score was significantly greater in *H. pylori*–infected rhesus monkeys than in either the uninfected or the GHLO-infected animals (Figure 5; $P < 0.05$).

Gastric metaplasia of the duodenum was not observed

**Figure 4.** Transmission electron micrograph of GHLO (original magnification ×18,000). Note that the bacteria are not closely associated with the epithelial cell surface and that microvilli are intact.

**Figure 4.** Transmission electron micrograph of GHLO (original magnification ×18,000). Note that the bacteria are not closely associated with the epithelial cell surface and that microvilli are intact.

**Table 1.** Natural History of *H. pylori* or GHLO Infections in Untreated Animals Over Time

| | Infection score | | | | | | |
|---|---|---|---|---|---|---|---|
| | *H. pylori* or GHLO (light microscopy)[a] | | *H. pylori* (culture)[b] | | Gastritis score[c] | | |
| Time (*mo*) | Corpus | Antrum | Corpus | Antrum | Corpus | Antrum | *H. pylori* IgG ratio[d] |
| *H. pylori*–infected (n = 6) | | | | | | | |
| 0 | 1.8 ± 0.5 | 2.3 ± 0.4 | 0.7 ± 0.2 | 1.0 ± 0.0 | 2.7 ± 0.3 | 3.0 ± 0.0 | 1.15 ± 0.28 |
| 7–15 | 1.2 ± 0.2 | 2.7 ± 0.1 | 0.8 ± 0.1 | 1.0 ± 0.0 | 2.2 ± 0.1 | 2.8 ± 0.1 | 1.66 ± 0.25 |
| GHLO-infected (n = 5) | | | | | | | |
| 0 | 2.8 ± 0.2 | 2.2 ± 0.5 | 0 | 0 | 0.4 ± 0.2 | 0.2 ± 0.2 | 0.41 ± 0.20 |
| 7–11 | 3.0 ± 0.0 | 2.4 ± 0.4 | 0 | 0 | 0 | 0 | 0.15 ± 0.06 |

NOTE. Values are means ± SEM for the corpus and antrum.

[a]Coded H&E-stained slides were scored for intensity of infection at 1000× using a scale of 0–3 (0, no bacteria; 1, colonies seen in 1–2 of 10 fields of view; 2, colonies seen in 3–8 of 10 fields; and 3, colonies seen in 9–10 of 10 fields).

[b]Homogenized biopsy specimens were cultured in microaerobic environment, and a score of 1 was given if colonies growing within 7 days were identified as *H. pylori* based on (1) pinhead-sized water-spray colonies positive for urease activity; (2) gram-negative curved or gull-wing rods therefrom; and (3) a kinetic assay showing high urease specific activity, in at least one culture from each rhesus monkey; otherwise, a score of 0 was given.

[c]Gastritis was rated independently from the scoring for infection on coded H&E slides using a scale of 0–3 (0, intact mucosal lining and essentially no infiltration of the lamina propria with lymphocytes and plasma cells; 1, mild increase of mononuclear infiltration, localized in upper half of the mucosa; 2, marked mononuclear infiltration extending from the surface to the muscularis mucosae; and 3, presence of polymorphonuclear leukocytes in glands, which was always associated with marked mononuclear infiltration and surface erosions).

[d]Plasma IgG levels were determined blindly using a modification of a previously described ELISA[29] using antimonkey IgG conjugates.

before treatment, and all 3 were negative for GHLO at 5 days. However, at 1 month, all 3 animals again had evidence for GHLO infection, which was present in an additional animal at 3 months. At 5 and 6 months, all 4 of these rhesus monkeys remained infected with GHLO, although each had been cleared of their *H. pylori* infection. Gastritis scores began to decrease 1 month after therapy; the score was ≤1.0 in all 4 rhesus monkeys in which *H. pylori* had been eradicated, whereas it returned to >2.0 in the 2 animals in which *H. pylori* infection relapsed (Figure 6). *H. pylori*–specific plasma IgG ratios decreased progressively after treatment in the animals in which infection had been eradicated, whereas it remained unchanged in the 2 animals in whom it was not eradicated (Figure 6). Thus, as in humans, both gastritis and specific immune response disappeared with eradication of *H. pylori.*

## Discussion

The present data show that infections with *H. pylori* and GHLO were enzootic in our colony. As in humans,[33,34] attachment of *H. pylori* to surface epithelial cells appeared to involve specialized receptors and pedestal formation (Figure 3*A*), and *H. pylori* organisms were rarely observed inside superficial epithelial cells (Figure 3*B*). In contrast, GHLO did not appear to adhere to surface epithelial cells (Figure 4),[10] and they were never seen inside surface epithelial cells, although they were often observed inside parietal cells.[21] In addition, contrary to our earlier series[21] and similar to the observation in humans[10,35] and in rhesus monkeys,[36] we found that *H. pylori* and GHLO could coexist in the same stomach, because 3 rhesus monkeys were proven to be infected with both types of bacteria by histological analysis and culture of *H. pylori.* In these 3 animals, however, the infection score for GHLO was significantly less than in animals infected with this bacterium alone (1.0 in all three animals vs. 2.3 ± 0.2), suggesting antagonism between *H. pylori* and GHLO. However, it is worthwhile noting that the diagnosis of GHLO rests entirely on light and electron microscopic examination of gastric biopsy specimens, because this bacterium has not yet been cultured. It is interesting to note that Reed and Berridge considered that GHLO were commensal organisms.[36]

*H. pylori* infection, as in humans,[37] was always associated with gastritis in the population of rhesus monkeys studied; this relationship persisted when infected animals were studied longitudinally. In addition, gastritis scores decreased over time in the four animals in which treatment eradicated *H. pylori* (Figure 6), thus indicating that, as in humans, gastritis is induced by *H. pylori* in rhesus monkeys. In contrast, animals apparently infected with only GHLO had minimal gastritis or none at all, which remained stable for months; this observation was confirmed in animals with persistent or new GHLO infection after clearance of *H. pylori.* Finally, gastritis and plasma

**Table 2.** Response of *H. pylori*–and GHLO-Infected Animals to Ineffective Triple Therapy

| Time (*mo*) | Infection score: *H. pylori* or GHLO (light microscopy)[a] Corpus | Antrum | *H. pylori* (culture)[b] Corpus | Antrum | Gastritis score[c] Corpus | Antrum | *H. pylori* IgG ratio[d] |
|---|---|---|---|---|---|---|---|
| *H. pylori*–infected (n = 2) | | | | | | | |
| −1 | 2.0 ± 0.7 | 3.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 3.0 ± 0.0 | 3.0 ± 0.0 | 2.75 ± 0.71 |
| 1 | 1.0 ± 0.7 | 2.5 ± 0.4 | 0.5 ± 0.5 | 1.0 ± 0.0 | 2.8 ± 0.2 | 3.0 ± 0.0 | 2.22 ± 0.26 |
| 3 | 1.5 ± 1.1 | 3.0 ± 0.0 | 0.5 ± 0.5 | 1.0 ± 0.0 | 2.0 ± 0.7 | 3.0 ± 0.0 | 3.25 ± 1.11 |
| GHLO-infected (n = 4) | | | | | | | |
| −1 | 3.0 ± 0.0 | 1.0 ± 0.6 | 0 | 0 | 0 | 1.0 ± 0.5 | 0.60 ± 0.27 |
| 1 | 2.3 ± 0.7 | 0.3 ± 0.2 | 0 | 0 | 0.3 ± 0.2 | 1.0 ± 0.4 | 0.50 ± 0.28 |
| 3 | 3.0 ± 0.0 | 1.0 ± 0.6 | 0 | 0 | 0.5 ± 0.4 | 0.3 ± 0.2 | 0.79 ± 0.38 |

NOTE. Values are means ± SEM for the corpus and antrum. Rhesus monkeys were treated with oral amoxicillin plus metronidazole plus bismuth subsalicylate (7, 7, and 10 mg/kg, respectively, three times daily) diluted in Tang for 4 weeks. All other footnotes are exactly as in Table 1.

IgG increased in the animal that became infected with *H. pylori* during the observation period, and the route of infection is at present unclear. Transmission during the endoscopies is possible, although unlikely, because we have taken stringent precautions and have cleaned the videoendoscope with glutaraldehyde and alcohol, rinsing and brushing the biopsy channel. Alternatively, oral-fecal transmission may have occurred in the rhesus monkeys of our colony, because recent publications have indicated that *H. pylori* may be isolated for the stools of ferrets[38] and humans.[39]

The mechanism by which *H. pylori* may cause gastritis is at present unclear. One possible explanation for this inflammatory reaction is that ammonium ions produced by bacterial urease activity[40] have a toxic effect on the gastric superficial epithelial cells. However, we observed that there was no such damage in animals harboring only GHLO, which are strong urease producers, as reported by Heilmann and Borchard[11] and in the present paper. This observation indicates that although urease is probably an important virulence factor that permits survival of these organisms in the gastric acidic environment, it does not necessarily produce gastritis in rhesus monkeys. Another possible cause of gastritis is the *H. pylori* cytotoxin, which has been shown to cause vacuolization in Hela cells[41–44] and may be responsible for the formation of intracellular vacuoles in gastric surface epithelial cells, immediately under the site of adhesion of *H. pylori.*[33] This effect may also be pertinent to rhesus monkeys because similar vacuoles have been observed in this species (Figure 3) and because *H. pylori* isolates cultured from the biopsy specimens of our animals produced the vacuolating cytotoxin at levels similar to those shown for isolates obtained in humans (T. L. Cover and M. J. Blaser, unpublished observations).

Alternatively, gastritis may be caused by antigen-mediated immunopathologic events that characterize this infection.[45,46] The specific immune response may be used as a diagnostic tool because gastric infection with *H. pylori* in humans is accompanied by elevated plasma levels of IgG and IgA.[29] In our study, IgG serology using an antimonkey conjugate was an accurate way to diagnose *H. pylori* infection. The discordance in two rhesus monkeys between high serological and inflammation scores and the inability to identify infection with *H. pylori* indicates that, as in humans,[47] serology may be more accurate than biopsy because it effectively samples the entire stomach. Among the rhesus monkeys studied, there was a significant positive correlation between *H. pylori*–specific plasma IgG level and gastritis score, suggesting that such levels may reflect immunopathogenetic events. In addition, the *H. pylori*–specific plasma IgG persisted over time in animals remaining infected; increased in the rhesus monkey that acquired infection during the observation period; and decreased over a 6-month period after eradication of *H. pylori* after effective therapy. Similar to the observation in humans,[48] the *H. pylori*–specific IgG levels initially decreased even in animals which subsequently relapsed. These findings confirm our previous observation that *H. pylori* organism isolated from the stomach of rhesus monkeys are antigenically related to human *H. pylori.*[21] In addition, they indicate that a modified *H. pylori* IgG ELISA using an antimonkey conjugate may allow the diagnosis of *H. pylori* infection in nonhuman primates, because it appears to reflect mucosal infection with these bacteria. Taken together, these observa-

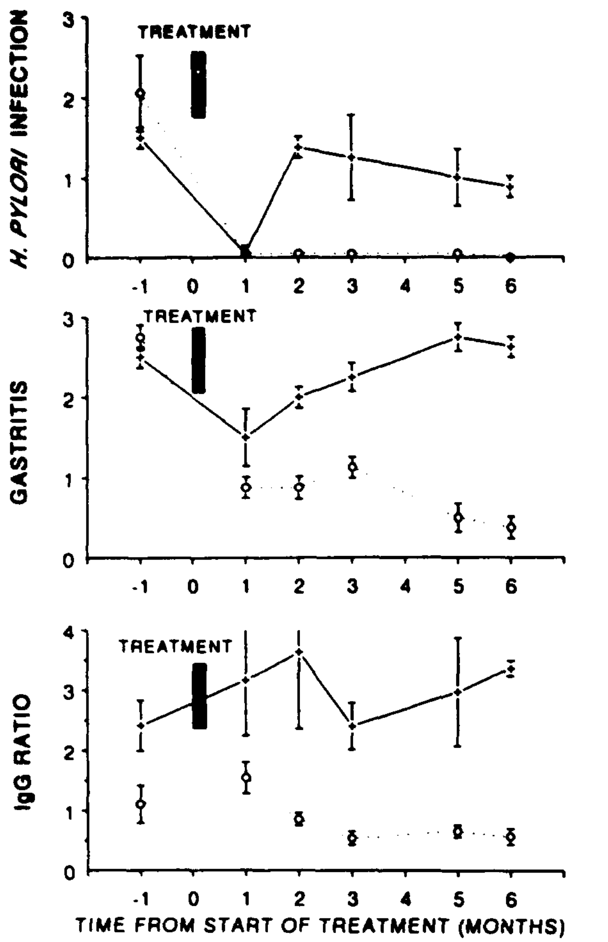


**Figure 6.** Effect of 10 days of intragastric administration of amoxicillin plus metronidazole plus bismuth subsalicylate (7, 7, and 10 mg/kg, respectively, diluted in distilled water, twice daily) on mean (±SEM) *H. pylori* infection scores, gastritis scores, and plasma IgG in two groups of *H. pylori*-infected rhesus monkeys. In four rhesus monkeys, infections were eradicated at 6 months (O); in two, infections relapsed before 6 months (+).

tions support the view that, as was shown in studies of humans,[47] measurement of plasma IgG levels against *H. pylori* may be superior to biopsy for evaluating the epizootiology of *H. pylori* infection in rhesus monkeys.

Among the 4 rhesus monkeys in which no infection with spiral bacteria was detected at the initial endoscopy, 1 animal had a gastritis score of 1.5 as well as an IgG ratio of 2.27; similarly high values persisted during the 4-month follow-up. Although gastric mucosal biopsy specimens from that animal remained negative for infection, these observations suggest that this rhesus monkey may have been chronically infected with *H. pylori*. Indeed, it is known that negative culture and histology do not exclude the presence of *H. pylori* in biopsy specimens from humans.[47]

Among the 9 GHLO-infected rhesus monkeys from which *H. pylori* could not be isolated, 8 animals had gastritis scores ≤ 1.5 and IgG ratio < 0.5. The remaining animal had gastritis scores of 1.0 and a plasma IgG ratio of 1.33, which suggests that a focal infection with *H. pylori* could have been missed because of the prominent GHLO infection at the site of the biopsies. In addition, GHLO infection may have suppressed *H. pylori* growth and viability in culture, leaving mucosal gastritis and an elevated IgG ratio as the only indicator of its presence in that particular biopsy. In humans infected with *G. hominis*, active chronic gastritis was either absent[24] or at least less severe than with *H. pylori* infection.[10,13,49] Furthermore, in these patients, endoscopic biopsy specimens were the only method used to rule out the simultaneous presence of both *H. pylori* and *G. hominis*, and *H. pylori*-specific serum IgG values were not measured. Additional studies will be needed to clarify the interactions between these two bacteria in the gastric mucosa.

The triple-therapy regimen developed for use in humans[50,51] initially decreased the level of *H. pylori* and GHLO infection in rhesus monkeys, but oral dosing with dilution in Tang did not achieve eradication of these bacteria. In contrast, this same regimen given intragastrically achieved clearance of *H. pylori* (but not of GHLO) in all 6 rhesus monkeys at 1 month and in 4 of 6 monkeys up to 6 months after treatment. The relapse observed in 2 rhesus monkeys could be caused by the fact that, as in humans,[52] rapid reinfection or incomplete eradication of these bacteria occurred, possibly as a result of resistance to metronidazole.[53]

In conclusion, the present studies indicate that gastric mucosal infection with *H. pylori* is common in rhesus monkeys and that, as in humans, this infection is associated with the presence of gastritis. In addition, serology allows noninvasive diagnosis of infection and of the response to antimicrobial therapy. Infection with GHLO is common in rhesus monkeys, whereas it is infrequently recognized in humans; however, the role of these organisms in inflammation appears to be low. Thus, naturally occurring *H. pylori* infection in this model may permit greater understanding of the transmission and pathogenesis of infection as well as the development and evaluation of new therapies.

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